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dietary linoleic and α-linolenic acids and their respective long-chain metabolites. Am. J. Clin. Nutr. *61,* 320–324.
52. Ratnayake, W. M. N., Sarwar, G. and Laffey, P. (1997) Influence of dietary protein and fat on serum lipids and metabolism of essential fatty acids in rats. Br. J. Nutr. *78,* 459–467.
53. Connor, W. E., Neuringer, M. and Reisbick, S. (1992) Essential fatty acids: The importance of n-3 fatty acids in the retina and brain. Nutr. Rev. *50,* 21–29.
54. Birch, E., Birch, D., Hoffman, D., Hale, L., Everett, M. and Uauy, R. (1993) Breast-feeding and optimal visual development. J. Pediatr. Ophthalmol. Strabismus *30,* 33–38.
55. Conquer, J. A. and Holub, B. J. (1996) Supplementation with an algae source of docosahexaenoic acid increases (n-3) fatty acid status and alters selected risk factors for heart disease in vegetarian subjects. J. Nutr. *126,* 3032–3039.
56. Grimsgaard, S., Bønaa, K. H., Hansen, J.-B. and Nordøy, A. (1997) Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids. Am. J. Clin. Nutr. *66,* 649–659.
57. Davidson, M. H., Maki, K. C., Kalkowski, J. A., Schaefer, E. J., Torri, S. A. and Drennan, K. B. (1997) Effects of docosahexaenoic acid on serum lipoproteins in patients with combined hyperlipidemia: a randomized, double-blind, placebo-controlled trial. J. Am. Coll. Nutr. *16,* 236–243.
58. Harris, W. S., Rambjør, G. S., Windsor, S. L. and Diederich, D. (1997) n-3 Fatty acids and urinary excretion of nitric oxide metabolites in humans. Am. J. Clin. Nutr. *65,* 459–464.
59. Hibbeln, J. R. and Salem, N. Jr. (1995) Dietary polyunsaturated fatty acids and depression: when cholesterol does not satisfy. Am. J. Clin. Nutr. *62,* 1–9.
60. Stevens, L. J., Zentall, S. S., Deck, J. L., Abate, M. L., Watkins, B. A., Lipp, S. R. and Burgess, J. R. (1995) Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorders. Am. J. Clin. Nutr. *62,* 761–768.
61. Martinez, M. (1996) Docosahexaenoic acid therapy in docosahexaenoic acid-deficient patients with disorders of peroxisomal biogenesis. Lipids *31,* 145S–152S.

# Radical Oxidation of Riboflavin

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*Summary: Riboflavin, lumiflavin and lumichrome were produced by light catalysis and gamma irradiation. Their formation under various conditions was determined, and a number of intermediates identified. Fluorescence excitation and emission spectra were determined for the compounds and compared with the absorbency spectra. While lumiflavin predominated in alkali and lumichrome in neutral solutions in the light-catalyzed reaction, all products were produced to some extent under all conditions. Gamma radiation resulted only in the formation of lumichrome, with no observable intermediates.*

## Introduction

Vitamins are the tissue components most sensitive to the gamma irradiation used to pasteurize foods [1]. Of the major vitamins in muscle tissue, thiamine and α-tocopherol are the most sensitive [1, 2], followed by riboflavin and niacin. The loss of these vitamins during the radiation pasteurization of meats has been extensively studied, but not the products formed. Because one of the important considerations in food irradiation is that toxic products are not produced in significant quantities, it is necessary to know the nature and quantity of the compounds produced from the vitamins by gamma irradiation. The loss of these vitamins is the result of oxidation by hydroxyl radicals produced by the reaction of a gamma photon with a water molecule. The light-catalyzed oxidation of riboflavin (7,8-dimethyl-10-(D-1'-ribityl)-isoalloxazine[1]) is also a radical reaction, the principal products of which are lumiflavin (7,8-dimethyl-10-methyl-isoalloxazine[1]) and lumichrome (7,8-dimethyl-alloxazine) [3–6] (Figure 1). Riboflavin and lumiflavin fluoresce greenish-yellow, with the $\lambda_{emission}$ ranging around 520 nm, typical of the isoalloxazine structure. Lumichrome fluoresces blue, with a maximum around 450 nm, typical of the alloxazine ring [4].

Much work has been done on these riboflavin oxidation products using the ultra-violet optical absorption of the compounds, but it has been shown that the optical absorptions of all the alloxazine derivatives are essentially the same for both the alloxazine and isoalloxazine electronic configurations [4]. Emission spectral maxima are shown in Table I [4, 7–9]. For purposes of identification of unknown compounds, optical absorption is not particularly useful for identification of the structures. Fluorescent identification, using either excitation or emission spectra has two advantages; it is more compound-specific and has a greater sensitivity than has ab-

1 In the latest numbering, the nitrogen in the ring to which the ribityl side chain is attached is the 10 position.

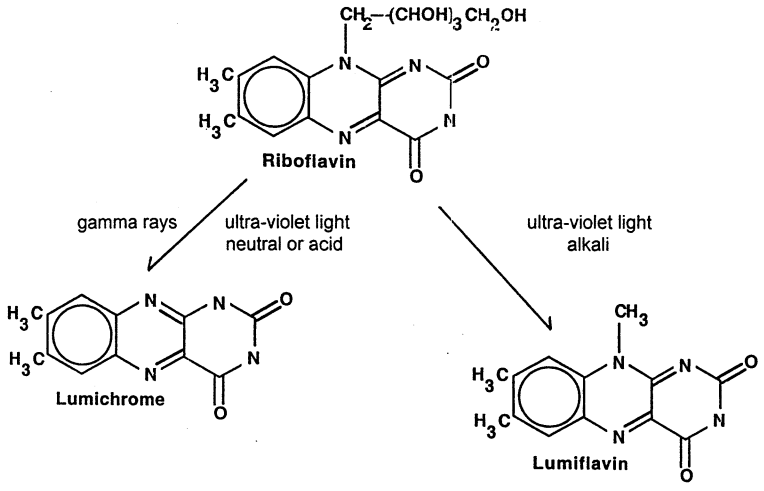


*Figure 1:* Structures of riboflavin, lumiflavin and lumichrome.

sorption. It has been shown that fluorescent emission spectra were unique to the given class of compounds, alloxazine or isoalloxazine [4]. The emission maxima of the isoalloxazines, riboflavin and lumiflavin, were reported to be $\lambda_{maxima}$ = 522 and 521 nm, respectively, and the emission maximum for the alloxazine, lumichrome, to be 462 nm.

There is some question as to the relationship of optical absorption to fluorescent excitation spectra, which are not necessarily the same. Although a number of emission spectra have been reported for riboflavin, lumiflavin and lumichrome (Table II), excitation spectra are rare. A study in which both absorption and excitation spectra were determined on the same compound reported excitation wavelength maxima at 270, 382 and 447 nm, and absorption maximal at 266, 359 and 433 nm [9]. In addition to the differences in the wavelength maxima, the relative intensities of the absorption and excitation maxima were different.

Fluorescent excitation and emission spectra are not necessarily always the same for different instruments. Whereas light absorption depends only on the relative amount of light transmitted compared to the incident light, fluorescent spectra are dependent on the spectral distribution of the excitation lamp, Raman light scattering and quenching effects [5]. The emission maxima are generally single Gaussian peaks, but excitation spectra, which are derived from the relative intensity of the emission maxima as the excitation wavelengths are scanned, will depend on the intensity of lamp emission at the various wavelengths. For this reason, we had to determine the excitation and emission spectra of riboflavin and its derivatives in our instrument. We prepared the various riboflavin oxidation products by light-catalyzed oxidation of riboflavin, chromatographed them, determined the excitation and emission spectra, and compared the results with the products of gamma radiation.

## Materials and Methods

Riboflavin (83-88-5) lot 109F0816, lumiflavin (1088-56-8) lot 28F3796, and lumichrome (1086-80-2) lot 57F3705 were purchased from Sigma Chemical, St. Louis, Missouri. All other chemicals were reagent grade. Water was glass-distilled and all other solvents were spectral grade. Riboflavin was irradiated in five different solutions: 50 mM acetate buffer, pH 5.5; $H_2O$:methanol::1:1; 0.1 NaOH; and water, with (pH 5.5) and without (pH 8.5) neutralizing the riboflavin.

*Light catalysis:* For the light-catalyzed oxidation, a 6×50 mm quartz mercury lamp (Model 6043, Oriel Corp., Stratford, CT) was immersed in the appropriate solution of riboflavin in a brown glass jar. Oxygen was slowly sparged through the solution to keep it saturated with oxygen and the riboflavin allowed to oxidize at different temperatures. For higher temperatures the jar was placed in a water bath dish on a hot plate. The temperature was monitored using an immersion thermocouple and the heater setting of the hot plate adjusted to maintain the desired temperature of the both at ±2°C.

*Table I:* Fluorescence spectra and retention times of riboflavin and its oxidation products

| Compound | Retention times (min) | Fluorescent maxima in nm Excitation | | | | | | | | | Emission |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Yellow fluorescence | | | | | | | | | | | |
| Riboflavin | 3.5 | 290 | 398 | 421 | 438 | 451 | 464 | **469**[1] | 475 | 482 | 525 |
| [5][2] | | | | | | | | | | | 531 |
| [4] | | | | | | | | | | 522 | |
| [7] | | **270** | 377 | | 447 | | | | | | 525 |
| Lumiflavin I | 1.4 | 260 | 364 | 420s | 439 | 450 | 462 | **470** | 473 | | 522 |
| (in $CHCl_3$) | | 260 | 365 | 420 | 440 | 451 | 465 | **469** | 475 | 483 | 507 |
| [4] | | | | | | | | | | | 521 |
| Lumiflavin II | 6.0 | | 370 | 421 | 439 | 451 | 464 | **468** | 475 | 487 | 516 |
| Blue fluorescence | | | | | | | | | | | |
| Lumichrome I | 2.5 | 294 | **364** | | | | | | | | 454 |
| Lumichrome (gamma) | 2.5 | 294 | **362** | | | | | | | | 450 |
| Lumichrome II | 10.5 | 265 | **358** | 398 | | | | | | | 461 |
| (in $CHCl_3$) | | 270 | **358** | 398 | | | | | | | 444 |
| [5] | | | | | | | | | | | 513 |
| [6] | | | | | | | | | | | 479 |

[1] Bold type values are the highest peaks in the spectra
[2] Number of the reference

*Gamma irradiation:* About 3 mL portions were poured into 13 × 100 mm tubes placed in a circular rack. The gamma irradiator has $^{137}$Cesium pencils arranged in an annular array around the radiation chamber and the circular arrangement of the samples produced an even dosage for the sets. The $^{137}$Cesium gamma radiation source (Lockheed-Georgia, Marietta, GA, Model LG. 20000) had a strength of 114 027 Ci (4.22 Pbq) and a dose rate of 0.101 kGy min$^{-1}$. The dose rate was established using National Physical Laboratory (Middlesex United Kingdom) dosimeters and corrected on a weekly basis for the decay of the isotope. Samples were maintained at 20 ±0.5°C during irradiation by thermostatically injecting gaseous nitrogen in the irradiation chamber.

*Chromatography and detection:* Riboflavin and its products were determined by fluorescence after chromatographic separation in a system similar to that used in a previous study in which a Cosmosil $C_{18}$ column was used [10]. Two tenths of a milliliter of a sample solution was injected on a Chrompak $C_{18}$ reversed phase column. The elutant per the previous study [10] was 64% water, 35% methanol and 1% 10% acetic acid ($H_2O$:MeOH:AcOH::64:35:1). After the chromatographic column, the elutant flowed first through a Waters 420-AC fluorometer set at $\lambda_{excitation}$ = 450 nm and $_{emission}$ = 530 nm (yellow, riboflavin and lumiflavin) and then through a McPerson FL-750 spectrophotofluorometer set at $\lambda_{excitation}$ = 365 nm and $_{emission}$ = 440 nm (blue, lumichrome). For the determination of the fluorescent spectra, the peaks were collected as they eluted from the column into 1 cm fluorescence cells and the solutions scanned for both excitation and emission spectra in a Perkin-Elmer MPF-44E fluorescence spectrophotometer equipped with a MPF-66 Xenon lamp. One advantage of fluorescent measurements for the determination of riboflavin and lumichromes is that the two excitation and emission spectra are separated by about 100 nm and 70 nm, respectively. Excitation of the fluorescence of lumiflavin at 365 nm produces no fluorescence of riboflavin or lumiflavin, and vice versa. It was possible therefore to determine the excitation and emission of the blue or yellow fluorescent compounds in the presence of the other fluorescent compounds, respectively.

*Table II:* Absorption spectra of riboflavin and its oxidation products

| | | Absorption Maxima in nm | | | | |
|---|---|---|---|---|---|---|
| Riboflavin | [7][1] | | **268**[2] | | 360 | 445 |
| | [8] | | | | 371 | 445 |
| | [9][3] | 210 | **270** | 344 | | **440** |
| | [10] | 223 | **267** | | 375 | 445 |
| | [4] | | 260 | 330 | 348 | 445 |
| Lumiflavin | [9][3] | | | 333 | | 440 |
| | [4] | | | | | 444 |
| Lumichrome | [9][3] | | | 329 | 382 | |
| | [4] | | **248** | 335 | 385 | |
| | [11] | | 262 | 350 | | |

[1] Number of the reference source of the data
[2] Bold type values are the highest peaks in the spectra
[3] Riboflavin in 98% dioxane, lumiflavin and lumichrome in pure dioxane

## Results

*Chromatography:* The chromatogram of the various compounds is shown in Figure 2, the retention times in Table I. All of the compounds were identified by their fluorescent spectra and by comparison with known compounds. The yellow fluorescent peak at 3.5 minutes was clearly riboflavin as it was the only peak in the initial solution. There were two lumiflavin (yellow fluorescence) and two lumichrome (blue fluorescence) peaks eluting at different times.

For sake of discussion we shall refer to them as lumiflavins I and II and lumichromes I and II. The two lumiflavin peaks and riboflavin had the same excitation and emission spectra. The excitation spectrum of the faster-eluting lumichrome I had a single excitation peak whereas lumichrome II had doublet peaks (Table I). Their emission spectra maxima were the same and occurred at shorter wavelengths than that of riboflavin or the lumiflavins. The elution sequence of lumiflavin I, riboflavin and lumichrome II is the same as earlier reported [10], although the retention times were somewhat different, probably because they used a different column. For purposes of identification, commercial preparations were chromatographed in our system. The lumiflavin from Sigma showed only one peak on chromatography, with a retention time of 6 minutes, the same as lumiflavin II. The Sigma lumichrome gave two blue fluorescing peaks at 2.5 and 10.5 minutes, the same as lumichrome I and II.

The two small peaks in the blue fluorescence scan at 4.0 and 6.5 minutes were light scattering peaks since the excitation maxima were the same as the emission maxima. Their intensities varied in different preparations, and did not change during the course of the oxidation.

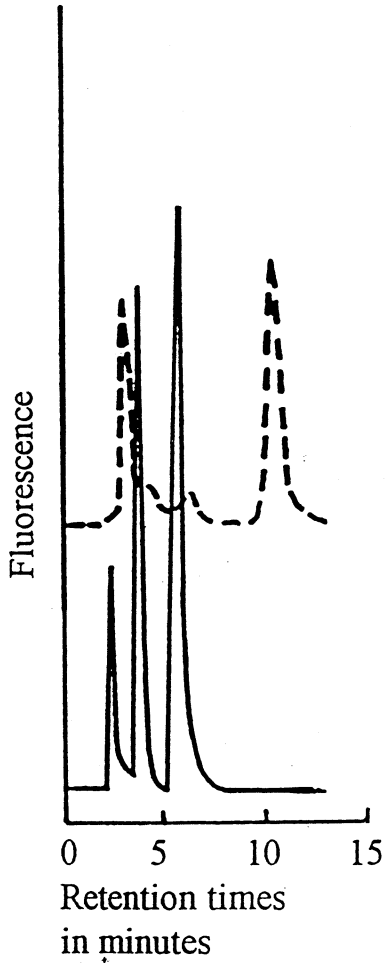


*Figure 2:* Chromatograph of riboflavin and its light-catalyzed oxidation products on Chrompak C18. Eluant, water:methanol:10% acetic acid::64:35:1. Dashed line, fluorescence at 440 nm. First peak, lumichrome I: next two low peaks, light scattering; last peak, lumichrome II. Solid line, fluorescence at 530 nm. First peak, riboflavin; second peak, lumiflavin I; third peak; lumiflavin II. Since the relative heights of the peaks vary during the course of the reaction, the heights shown are for illustrative purposes only.

*Reaction sequence:* The fluorescent peak heights of all four products increased during the oxidation as the riboflavin fluorescence decreased. Lumiflavin II was produced at a faster rate than was lumiflavin I, but as the reaction proceeded and the riboflavin concentration was reduced to zero, the lumiflavin I peak continued to rise, while the lumiflavin II peak went down, that is, the latter was a precursor of the former.

Lumichrome II was produced to varying extents in all solutions but to a lesser extent in alkaline solutions where the final product was lumiflavin I. As with the lumiflavins, lumichrome II was initially produced in larger quantities than lumichrome I. The quantity of lumichrome II produced during the reaction decreased with increasing temperature and was not seen at all above 75°C. Lumichrome II was highly unstable to handling. It was soluble in chloroform, but when the chloroform solutions were dried down in an attempt to concentrate the solution, upon dissolution in water:methanol::1:1, it converted to lumichrome I, coming off the column at 2.5 minutes.

If the ultra-violet light irradiation process was allowed to run for a long time (several days), both the lumiflavin II and lumichrome II peaks completely disappeared, with a net increase in lumiflavin I and lumichrome I.

*Excitation and emission spectra:* Three types of excitation spectra were observed (Figure 2), a

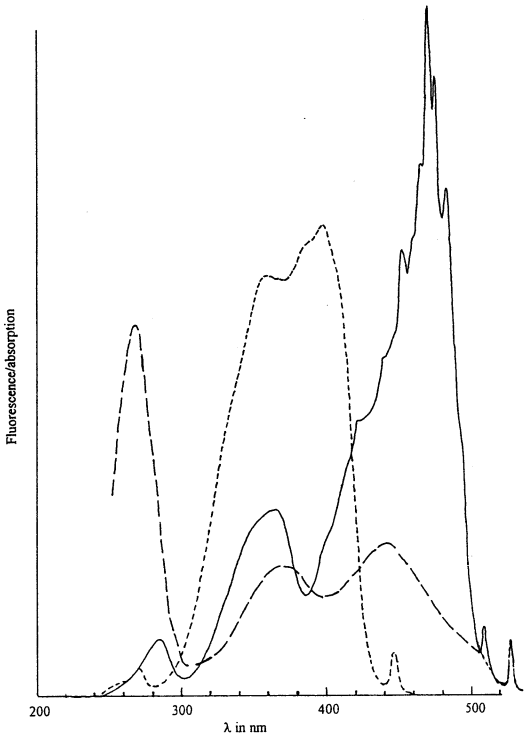


*Figure 3:* Fluorescent excitation spectra of riboflavin/lumiflavin (solid line) and lumichrome II (dotted line); absorption spectrum of riboflavin and its derivatives (dashed line). Spectra are tracings from recordings of specific compounds typical of the class and are not necessarily the same scale of intensity. Ordinate is intensity of emission at 525 nm (solide line) and 445 nm (dotted line), as well as intensity of absorption (dashed line). Low peaks at 445 nm and 525 nm are the light scatter peaks as the fluorometer excitation light scanned past that wavelength.

multiple peaked curve for riboflavin and lumiflavin, a single smooth peak for lumichrome I (not shown) and a doublet for lumichrome II (shown). A typical absorption spectrum [9] is shown for comparison and it is readily apparent that the light absorbing electron structures in the molecule that produce fluorescence are not quite the same as the electronic structures responsible for the optical absorption. The emission spectra of all compounds were single, broad, symmetrical peaks and are not shown.

*Reaction environment:* While in general the oxidation products were as reported in the literature, lumiflavin in alkali and lumichrome at lower pH values, all of the compounds were produced in varying quantities at all pH values. While the major product in alkali (pH ~12.5) was lumiflavin, there was an appreciable production of lumichromes I and II. At lower pH values, the amounts of lumichromes I and II produced were about equal, with lesser amounts of lumiflavin. There was no effect of either methanol or acetate on the light-catalyzed reaction.

*Gamma radiation of riboflavin:* While the light-catalyzed oxidation of riboflavin proceeded quite slowly at room temperature, the oxidation by gamma radiation went quickly at fairly low doses at 20°C. In water or alkali the riboflavin was completely oxidized at 2.5 kGy. In contrast to the light-catalyzed reaction, the only product seen was lumichrome, identified by both its retention time and fluorescent spectra (Table I). There were several small blue fluorescent peaks in the initial riboflavin solution, but they all disappeared at 0.1 kGy. After peak production of lumichrome at 1.0 kGy, it too was destroyed and was gone by 6.0 kGy. The rates of loss of both riboflavin and lumichrome were the same in water and in alkali (Figure 3). Lumiflavin was not observed under any conditions.

In methanol, an increasing amount of measured riboflavin up to about 0.2 kGy was observed, after which it was slowly destroyed. This phenomenon has been observed previously in irradiated meats [1], and is a function of the presence of an organic molecule since the other solutions did not show the increase during irradiation. This destruction was not accompanied by the formation of either the lumiflavins or lumichrome I, although small amounts of lumichrome II were observed during the course of the reaction.

## Discussion

The excitation and absorption maximum wavelengths show the two light absorption processes are not the same. While the wavelength of maximum optical absorption is in the 250 to 260 nm range, this wavelength had little effect on excitation of the fluorescence of the molecule. This is in contrast to previous results [9] in which the reported excitation spectra show

268 nm peaks five times the heights of the peaks at 360 and 445 nm. The problem is that fluorescence is a secondary phenomenon in which the amount of emitted light is dependent on the intensity of the exciting light source. A Perkin-Elmer LS-5B was used in that study [9], and had they used a lamp with a higher intensity in the 260–280 region than the Xenon lamp we used, they would have perforce reported a higher peak in the 260–280 nm range than we observed.

The excitation spectra of the isoalloxazines, riboflavin and lumiflavin, are highly complex, with a series of narrow peaks, with a maximum peak at 468–470 nm. The number of small peaks indicate a number of electron excitation levels that can contribute to the fluorescence. This may be a consequence of the strained configuration of the alloxazine ring due to the substitution on the 10 position of the ring. The smoother peaks of the alloxazine lumichromes indicate a freer electron configuration. The doublet peak of lumichrome II would seem to indicate two separate compounds, but there was only one emission maximum and we observed no indication of a doublet peak in the chromatogram. The excitation spectra we obtained in the MPF-44E are different from those reported in the literature [9] in which two excitation maxima at 377 and 447 nm were reported for riboflavin, without the fine peak detail we obtained on the MPF-44E. Since the excitation spectra of the blue fluorescent peaks under the same conditions of recording did not show the fine peak detail, we conclude that the fine details in the riboflavin spectra were not artifacts of the MPF-44E.

There are two electronic excitation bands common to the absorption spectra and the excitation spectra of the riboflavins, the bands at 360–380 and 438–447 nm. The 438–447 absorption band is due to the electronic transition between the highest filled and lowest empty orbitals of riboflavin [12]. For lumichrome, the calculated wavelength for the highest filled and lowest empty orbitals was 382 nm [12] which is sufficiently close to the excitation wavelength maxima (362) to conclude they are due to the same electron transition. These two transitions represent the highest energy transfers in the molecules, yet maximal fluorescence excitation was observed at wavelengths of both higher and lower energy. This means that the electron transition resulting in light emission was between lower energy levels, and that electrons excited to higher levels dropped down to the lowest empty orbital before the light emitting transition took place.

*Lumiflavin II and lumichrome II:* As noted previously, the reaction behavior of both of these two compounds mark them as precursors of lumiflavin I and lumichrome I. Lumichrome II is the compound that previously has been designated as lumichrome [10]. Lumiflavin I has been designated as lumiflavin [10], but the Sigma lumiflavin corresponded to lumiflavin II. As of now we have no evidence as to which is the basic compound since the only observed differences are their retention times. Lumichrome II is probably the same as compound X [6] with which it shares many of the same characteristics.

*Gamma irradiation:* While the light-catalyzed reaction produced at least three identifiable pigments, the gamma radiation oxidation produced only lumichrome I. That lumiflavin was not seen shows that the two oxidation processes are not the same. The light-catalyzed reaction is the result of the excitation of the electronic structure of riboflavin, and takes at least two different reaction pathways depending on where the ultraviolet light excitation results in the most reactive site. In contrast, the gamma radiation oxidation is driven by the very reactive hydroxyl radical produced by the reaction of gamma photons with water. Such a reaction would be expected to be homogeneous since the electronic configuration of the alloxazine ring contributes little to the energetic requirements of the reaction. That the energy for the reaction derives primarily from the gamma photon is demonstrated by the observation that while lumiflavin and lumichrome are only slowly oxidized by continued exposure to light, gamma radiation oxidized the lumichrome almost as fast as was riboflavin. A direct interaction between the gamma photons and the riboflavin ring is possible, but the probability of reaction of the photon with water is several million times that of a direct interaction with the riboflavin molecule at the concentrations used in this study.

## Conclusion

The gamma radiation oxidation of riboflavin, while it is not the same process as the light-catalyzed oxidation, results in lumichrome which is one of the products of the latter reaction. Since lumichrome has been found to occur naturally in milk [10], in pasta exposed to light [15] and in the retina of the eye [16], it is not unique to the gamma irrradiation process. Furthermore, since the loss of riboflavin is very low in gamma-ray pasteurization of meats [1], the amount produced is negligible and probably constitutes no significant health hazard. There is some uncertainty in the literature as to the identity of these compounds, probably because previous workers have been looking at one or the other of the two chromatographically distinct forms of lumiflavin and lumichrome.

## References


1. Fox, J. B. Jr., Thayer, D. W., Jenkins, R. K., Phillips, J. G., Ackerman, S. A., Beecher, G. R., Holden, J. M., Morrow, F. D. and Quirbach, D. M. (1989) Effect of gamma irradiation on the B. vitamins of pork chops and chicken breasts. Int. J. Radiat. Biol. *55,* 689–703.
2. Lakritz, L. and Thayer, D. W. (1992) Effect of ionizing radiation on unesterified tocopherols in fresh chicken breast muscle. Meat. Sci. *32,* 257–265.
3. Karrer, P., Salomon, H., Schöpp, K., Schlittler, E. and Fritzsche, H. (1934) Lumichrome, a new irradiation product of lactoflavin. Helv. Chim. Acta *17,* 1010–1013.
4. Berezovskii, V. M. and Aksel'rod, Zh. I. (1966) Study of the structure of alloxazine N-oxides by electronic and fluorescence spectroscopy. Doklady Akademii Nauk SSSR *71,* 1101–1104.
5. Ellinger, P. and Holden, M. (1944) Quenching effect of electrolytes on the fluorescence intensity of riboflavin and thiochrome. Biochem. J. *38,* 147–150.
6. Smith, E. C. and Metzler, D. E. (1963) The photochemical degradation of riboflavin. Biochem. J. *85,* 3285–3288.
7. Yagi, K., Tabata, T., Kotaki, E. and Arakawa, T. (1995) Fluorometric analysis of vitamins II. Fluorescence spectra. Vitamins-Kyoto *8,* 61–63.
8. Kamin, H. (1971) Flavins and flavoproteins: Proceedings of the third international symposium on flavins and flavoproteins Durham NC. University Park Press, Baltimore, MD.
9. Magee, J., Kraynack, N., Massey, H. C. Jr. and Telfer, W. H. (1994) Properties and significance of a riboflavin-binding hexamerin in the hemolymph of *Hyalophora cecropia.* Arch. Insect Biochem. Physiol. *25,* 137–157.
10. Toyosaki, T. and Hayashi, A. (1993) Structural analysis of the products of milk riboflavin photolysis. Milchwissenschaft *48,* 607–609.
11. Weber, G. (1950) Fluorescence of riboflavin and flavin-adenine dinucleotide. Biochem. J. *47,* 114–121.
12. Koziol, J. (1965) Absorption spectra of riboflavine, lumiflavine and lumichrome in organic solvents. Experientia. *21,* 189–190.
13. Daglish, C., Baxter, N. and Wokes, F. (1948) Spectroscopy of riboflavin. Quarterly J. of Pharmacy and Pharmacology *21,* 344–355.
14. Foster, J. W. (1944) Microbiological aspects of riboflavin. I. Introduction. J. Bact. *47,* 27–41.
15. Woodcock, E. A., Warthesen, J. J. and Labuza, T. P. (1982) Riboflavin photochemical degradation in pasta measured by high performance liquid chromatography. J. Food Sci. *47,* 545–549.
16. Brunner, O. and Baroni, E. (1936) Retinal Substances. IV. The flavins of the retina. Monatsh. Chem. *68,* 264–273.